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(71) Applicant and
(72) Inventor: **HOLLEY, John, Ernest, Foster** [GB/GB]; 9 Shipfield Close, Tatsfield, Near Westerham, Kent TN16 2AU (GB).

(74) Agent: **COHEN, Alan, Nicol**; 2 Grove Place, Tatsfield, Near Westerham, Kent TN16 2BB (GB).

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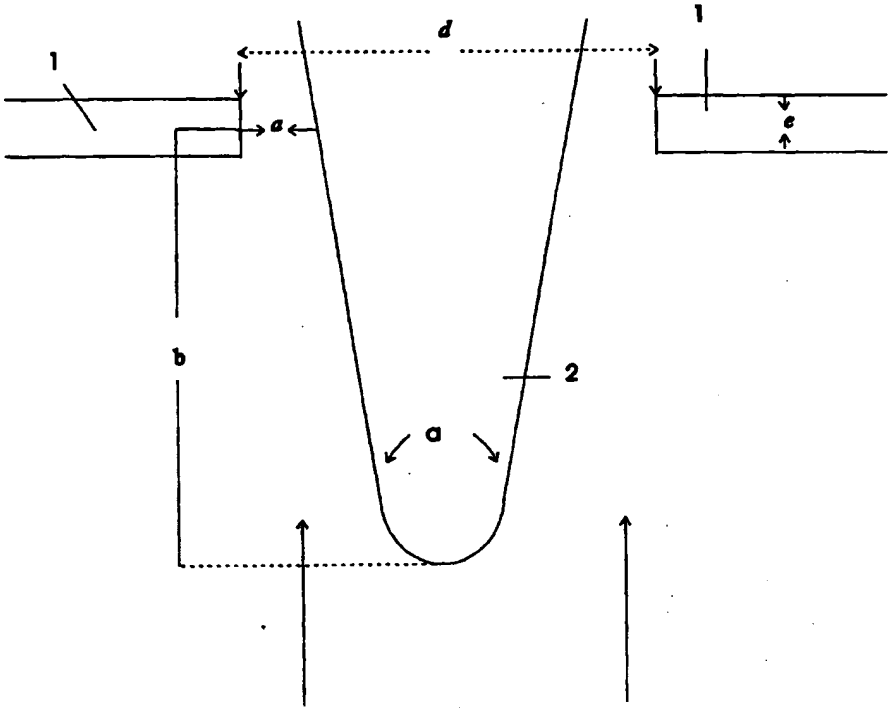
(54) Title: SEPARATION METHOD



(57) Abstract: A device for separating particles in liquid suspension comprises a circular orifice with an insert so as form an annular gap, it is particularly useful in separating agglutinated particles and the device can also be used to count particles.

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Separation Method

This invention relates to an improvement in a method of separating small particles and for analysing particles in liquid suspensions.

Our European Patent Application No. 0242971 describes a scanner element for use in a Coulter type counter in which element there is an orifice through which the liquid can pass and there is a moveable insert which can be moved in and out of the orifice to vary the cross-sectional area through which the liquid can pass. This patent application illustrates devices in which the insert is tapered or has its cross-sectional area varied in steps.

By a better understanding of the phenomena occurring in liquid flow through annular orifices, we have now devised improved systems. It has surprisingly been discovered that by a selection of specific dimensions of the components of the system, enhanced filtration/separation performance can be obtained.

It has also been found that this scanner element facilitates separation processes which have been difficult to carry out hitherto.

According to the invention, there is provided a filtration device, which device comprises a substantially circular orifice through which liquid can pass, there being an insert mounted within the circular orifice to form an annular orifice, the insert projecting beyond the end of the circular orifice for a distance such that ratio of the width of the annular orifice to the distance the insert projects beyond the circular orifice is from 1: 10 to 1:500, preferably 1:20 to 1:250.

The insert can be tapered and moveable in and out of the circular orifice to provide an annular orifice of variable size. In this case the angle of the taper should be less than

8° and preferably 4 ° -8 °. By angle of the taper is meant the angle formed between two lines on opposite sides of the insert projected, if necessary, beyond the end of the insert.

Preferably the ratio of the width of the annular orifice to the length of the substantially circular orifice is less than 4:1 and can be below 1:1; in practice the orifice can be almost closed by the insertion of the insert, in which case the ratio can be as low as 1: 10 or less.

We have found that the improved filtration/separation effects are obtained if the width of the annular orifice is below about 40 microns.

In use, liquid containing the particles to be separated is passed through the device so that the liquid passes over the projecting portion of the insert before passing through the orifice and we have found unexpectedly that there is very effective prevention of particles above a specific size from passing through the annular orifice, even though these particles are smaller than the orifice and would have been expected to pass through. Also there is substantially very little blocking of the annular orifice, even though quite "sticky" organic or biological particles are present.

The effect is such that for a given size of orifice there is a nearly complete separation of particles above a specific size.

The mechanism for this surprising effect is not understood but it is thought the effect is similar to if a standing wave is set up which diverts the larger particles or spins them away from the annulus by some other mechanism. Alternatively or additionally, there could be two forces that act together. One force is the edge effect, which allows some particles to be attracted towards the insert and are drawn up past the annulus. the second force is laminar flow, which will take the larger particles away from the annulus.

The flow of liquid through the device should preferably be laminar and a flow rate low enough to prevent turbulence should be used and we have found that a pressure of below about 8" of mercury is preferred.

The end of the insert projecting out is preferably curved or smooth or of a shape which reduces the likelihood of turbulence as liquid passes over it, e.g. in the shape of a tapered round column.

Insertion of the insert into the substantially circular orifice will decrease the width of the annular orifice and will alter the flow characteristics of liquid flowing through the annular orifice and this can enhance the filtration effect.

The device of the invention can be made of any material which is inert to the liquid being passed through it.

The device of the invention is useful for separating particles from bio-active systems such as cells where particles are typically in a size range of 0.1 to 30 microns and it is useful in separating and quantifying particles below a specific size.

However, the device of the invention can be used in any system where it is desired to remove particles above a particular size in the appropriate range, e.g. up to about 100 microns.

For larger flow rates, a module can be made or formed of a plurality of the devices of the invention joined together to cover a larger cross-sectional area. In this case normally the inserts would be fixed in position and the module would act as an efficient precise filter.

A particular application of the present invention is for separating agglutinated from non-agglutinated particles.

It is known to take beads of e.g. 0. 1 to 30 microns diameter and to coat them with an antigen or antibody. When these coated beads are placed in contact with a liquid containing material specific to the antigen or antibody, this specific material will attach itself to the coated beads. In this way the coated beads can be joined together by mutual adhesion to the specific material and this is commonly referred to as agglutination.

The beads can be made of glass, latex, polystyrene or other material known or used for this purpose. If it were possible to determine how many beads were agglutinated this would be a measure of the concentration of the specific material in the liquid to which the coated beads are added.

It has been found that the bond binding the beads together is weak and it has been suggested to filter out or separate the agglutinated beads from the other beads; however previous attempts, e.g. using filter techniques, leads to a break-up of the agglutinated beads.

The use of the device of the present invention is an effective method for separating agglutinated from non-agglutinated particles.

It has been found that for particles in the range of 4 μm to 40 μm which have a diameter smaller than the width of the annular gap are prevented from reaching the gap and are diverted away from it.

Particles of a diameter substantially below the width of the annular gap can pass through the gap relatively easily, thus it is possible to separate the agglutinated beads from the non-agglutinated beads.

This diversion of the larger particles has a relatively reduced shearing effect compared to conventional filtration and can be used to separate agglutinated particles from non-agglutinated particles.

If a liquid is thought to contain a specific bio-active material, its concentration can be determined by adding to the liquid a known number of beads coated with an antigen or antibodies to the bio-active material to give a known concentration of coated beads.

After a known time, to allow for the beads to agglutinate, the agglutinated beads are separated from the non-agglutinated beads and the concentration of non-agglutinated beads are determined. By this means it is possible to determine the concentration of agglutinated beads in the liquid and hence the concentration of the bio-active material. The volume of the sample containing the coated beads should preferably be kept as low as possible as this will reduce the distance between the beads and the reactant, thereby increasing the number of collisions or contacts between the beads and the reactant in a given time and thus increasing the number of beads to which the reactant adheres.

Preferably, before being passed through the orifice, the liquid containing the agglutinated and the non-agglutinated beads is diluted for ease of operation e.g. with a diluant salt solution.

Another application of the present invention is for detecting and counting live micro-organisms, particularly when mixed in liquids with dead micro-organisms or other particles in the same size range.

It is known that live micro-organisms can be impregnated with a substance which causes a vital stain which, when exposed to a specific waveband of light, particularly ultra-violet light will fluoresce at a second waveband, this is known as a Stokes Shift.

Methods have been proposed for detecting these micro-organisms by shining a strong beam of light of appropriate frequency through a liquid containing these microorganisms and detecting and counting the fluorescing micro-organisms.

However, it has been found that, in order to obtain a strong enough signal to make detection practical, an incident beam is required, such as a laser, which has an intensity which is likely to damage micro-organisms present and would cause there to be so much reflected light that it would make detection of the relatively weak fluorescence difficult.

If the insert is made of an optically transparent insert material whereby liquid flowing through the orifice can pass over the insert, and light is transmitted through liquid and fluorescence of fluorescable particles in the liquid passing over the insert can be detected.

By fluorescable particles is meant particles which fluoresce when exposed to ultra-violet light.

Preferably they emit light of 300-700 μm when they fluoresce. In some applications, particles which would not otherwise fluoresce, can be contacted with a suitable fluorescent pigment which would adhere to them and thus make them detectable. Coherent light produced by a suitable light source (e.g. a xenon lamp, a deuterium lamp or a laser diode) is preferably used as these can be arranged to give a beam with a very narrow wavelength.

For use in the detection of living cells and micro-organisms, the orifice preferably has a diameter of between 60 and 200 μm, more preferably of between 70 and 100 μm.

The orifice can be formed in any suitable inert material such as glass quartz, ruby, plastic, sapphire or other material, using known methods.

The insert is preferably made of optical quality glass and is constructed of two different glasses of different refractive indices, so that one glass forms a sheath around the other glass in order that total internal reflection takes place for light passing down the insert. The light is then preferably conducted away from the insert by means of a conventional light guide.

The insert is preferably placed as close to the orifice as possible without affecting the flow of liquid through the orifice to an unacceptable degree. Preferably the insert has a substantially flat end so that light emitted from the particles as they pass the insert can pass into the end of the insert, in some cases, the particles may hit the end of the insert.

In one embodiment of the invention the light can pass down the insert into the liquid and the fluorescent light emitted by the particles can then pass back up the insert. By suitably positioning the insert in relation to the size of the orifice, substantially all the particles in the liquid are caused to pass near enough to the end of the insert so that they receive light at a strength sufficient to cause the fluorescable particles to fluoresce.

In an another embodiment of the invention the light from a narrow bandpass source can pass across the end of the insert and the fluorescent light then passes into the insert.

Preferably the fluorescent light emitted by the fluorescable particles passes up through the insert via filters to remove any reflected ultra-violet light before going to a detector and counter. Conventional detectors and counters can be used such as a single photon counting module, e.g. model SPCM- 1 00-PQ, made by General Electric Canada Inc. Modulation of the incident light will also assist in the ability to read the low level output of the fluorescent particles.

Live particles, such as cells or micro-organisms, fluoresce at a different wavelength to dead particles so, by counting the number of particles which fluoresce at different wavelengths, the ratio of dead to live particles can also be obtained.

It is a feature of the present invention that particles pass very close to the insert and consequently the fluorescent light, which inevitably is considerably weaker than the incident light, only has a short distance to travel through the liquid to the insert. This feature enables lower power light sources to be used to provide the incident light.

The present invention enables particles to be counted accurately as they pass through the orifice and so it enables the concentration of the fluorescable particles in the liquid to be obtained, even when mixed with other particles in the same size range. The outputs from the light detector can be fed to a recording device or into a computer for further processing.

The invention is now described with reference to the accompanying drawings, in which :-

Fig. 1 is a side view of a device of the invention

Fig. 2 is a crosssectional view of the device viewed along the insert.

Fig. 3 shows the light path from orifice to detector and light source

Fig. 4 is a side view of the device in solution

Fig. 5 is a side view of the orifice and input light source

Referring to figs. 1 and 2 a plate (1) has a circular orifice (3) of diameter (d) which is 70 microns, a tapered insert (2) fits within the orifice (3) to leave an annular orifice (4) of width (a). The insert (2) projects a distance (b) beyond plate (1). The angle a is the angle of taper of the insert. The orifice was formed in a synthetic ruby and the insert (A) was formed in quartz glass.

0.1 % salt diluant solution containing latex beads of varying sizes were passed through the device in the direction of the arrow and the filter effect measured. In all cases no blocking of the element occurred.

The length of the circular orifice (3) shown as (e) was 25 microns and a was 5 degrees.

Table

a microns	b microns	Size of Particles Separated Microns
2	500	1
4	450	2
5	400	4.5
8	300	6
10	200	10

This shows that, at small values of a, particles smaller than the dimension of a are prevented from passing through the annular orifice, thus causing more efficient separation.

Referring to fig. 3 a glass insert (12) of diameter 30-100 μm is positioned adjacent to the orifice (11) with a gap between the end of the orifice and the plate (17) of a range of 2 - 400 μm, so that liquid can pass over the orifice.

Light guide (13) made of glass cable is attached to the insert (12) so that light will pass from the insert down the light guide and vice versa. The light guide (13) bifurcates to form two arms (14) and (15). The arm (14) goes to a source of ultra-violet light (25) and the arm (15) goes to a filter (16) which filters out reflected ultra-violet light and then bifurcates again into two arms (27) and (28). The arm (27) has a filter to remove red light and the arm (28) has a filter (20) to remove green light. Arms (27) and (28) then go to light counters (21) and (22) respectively, which are single photon counting modules No. SPCM- 100 PQ made by General Electric Canada Inc.

In use, liquid containing particles go through orifice (11) into and over insert (12). Ultra-violet light passes down light guide (14), strikes the particles and reflected fluorescent light and passes back down insert (12). Some of this reflected light passes along light guide (15) where ultra-violet light is filtered out by filter (16). Some of this light passes down light guide (27) and through filter (19) which only allows green light to go to counter (21). Light passing down light-guide (28) passes through filter (20) which only allows red light to go to counter (22).

Live particles will fluoresce in the red part of the spectrum and the light they emit will go to counter (22), which counts such particles as a pulse of red light. Dead particles will fluoresce in the green/yellow part of the spectrum and the light they emit will go to counter (21), which counts such particles as a pulse of green/yellow

light. By comparing the number of pulses of light counted by counters (21) and (22), a ratio is obtained of live to dead cells.

This counting is done automatically by counters (21) and (22) and, if required, their output can be fed directly into a recording device, either as separate counts or as a ratio. Alternatively, instead of separate filters (19) and (20), a single filter, which can be rotated to filter out sequentially red and green light, can be used. In this case, only one photon counting module is required.

Referring to fig. 4 a container (33) contains liquid in which there are the particles which are to be detected and counted.

A holder (32) consists of a vessel which has an orifice (23) of about 70μm diameter in its bottom. There is a "spear" (24) made of glass which has a diameter of its end (26) of 60μm. The spear (24) can be moved nearer and further from the orifice. Light collimator or guide (29) is able to pass light from light source (27) through filter (28) across the orifice (23) as shown by the arrows.

The spear (24) can conduct light reflected by particles passing across orifice (23) through filter (31) to detector and counter (32). Conduit (25) is connected to a vacuum pump so that liquid can be drawn through the orifice (23).

In use liquid containing the particles or organisms it is desired to detect and count is drawn through the orifice (23) by the action of the vacuum pump connected to conduit (25).

Light of the appropriate wavelength to excite the particles or organisms to fluoresce, normally ultra violet light, passes down light guide (29) strikes the particles and reflected fluorescent light passes back down spear (26), the ultra violet light is filtered out by filter (31) which can also filter out light of other wavelengths.

Live particles will fluoresce in the red part of the spectrum and the dead particles will fluoresce in the green/yellow part of the spectrum and thus with the appropriate detectors it is possible to count the number of dead and live particles. Instead of red and green filters other coloured bandpass filters can be used as required.

Referring to fig. 5 which is an enlarged diagrammatic view of the invention, the orifice bounded by the two sides (42a) and (42b) and liquid containing particles is drawn through the orifice. Light passes down the light guide (41) as shown by the arrows and emerges to illuminate the particles in the liquid. When this light strikes an organism as shown by (44) which fluoresces the fluorescent light formed passes down the end of the probe or spear (43) as shown by the arrows.

Claims

1. A filtration device which comprises a substantially circular orifice through which liquid can pass, there being an insert mounted within the circular orifice to form an annular orifice.

2. A filtration system as claimed in claim 1 in which the insert projects beyond the end of the circular orifice for a distance such that ratio of the width of the annular orifice to the distance the insert projects beyond the circular orifice is from 1: 10 to 1:500

3. A filtration device as claimed in claim 2 in which the ratio of the width of the annular orifice to the distance the insert projects beyond the circular orifice is from, 1:20 to 1:250.

4. A filtration device as claimed in claim 2 or 3 in which the insert is tapered and moveable in and out of the circular orifice to provide an annular orifice of variable size.

5. A filtration device as claimed in claim 4 in which the angle of taper is less than 8^{o}.

6. A filtration device as claimed in claim 4 in which the angle of taper is 4^{o} -8^{o}.

7. A filtration device as claimed in any one of claims 2 to 6 in which the ratio of the width of the annular orifice to the length of the substantially circular orifice is less than 4:1.

8. A filtration device as claimed in any one of claims 2 to 6 in which the ratio of the width of the annular orifice to the length of the substantially circular orifice is less than 1:1.

9. A filtration device as claimed in any one of claims 2 to 6 in which the ratio of the width of the annular orifice to the length of the substantially circular orifice 1:10.

10. A filtration device as claimed in any one of claims 2 to 6 in which the width of the annular orifice is below 40 microns.

11. A filtration device as claimed in any one of claims 2 to 6 in which the insert is optically transparent, there being means to transmit light through the liquid and means to detect fluorescence of fluorescable particles in the liquid passing over the insert.

12. A device as claimed in claim 1 in which light is transmitted down a light guide to the insert and light emitted by particles fluorescing is transmitted back up the insert to a detector.

13. A device as claimed in claim 12 in which the insert has a substantially flat end so that light emitted from the particles as they pass the insert passes into the end of the insert.

14. A device as claimed in any one of claims 12 or 13 in which there is a light conducting means adapted to pass light down the insert into a liquid flowing through the orifice and a light conducting means adapted to pass light emitted by the particles back up the insert.

15. A device as claimed in any one of claims 12 to 14 in which the insert can be positioned in relation to the orifice so that, in use, substantially all the particles in a liquid passing through the orifice pass near enough to the end of the insert so that they receive light at a strength sufficient to cause the fluorescable particles to fluoresce.

16. A device as claimed in any one of claims 12 to 15 in which there are band pass glass filters able to remove reflected ultra-violet light positioned such that fluorescent light emitted by the fluorescable particles will pass through the filters before going to a detector and counter.

17. A device as claimed in any one of claims 12 to 16 in which the orifice has a diameter of between 60 and 200 μm.

18. A device as claimed in any one of claims 12 to 16 in which the orifice has a diameter of between 70 and 100 μm.

19. A device as claimed in any one of claims 12 to 18 in which the orifice is formed in glass quartz, ruby or sapphire.

20. A device as claimed in any one of claims 12 to 19 in which the insert is made of optical quality glass and is constructed of two different glasses of different refractive indices with one glass forming a sheath around the other glass.

21. A method for separating larger particles from smaller particles which method comprises passing a liquid containing the different sized particles through an orifice which has an insert placed within it so as to form an annular gap of a size which prevents particles of above a predetermined size from contacting the gap and passing through it.

22. A method as claimed in claim 21 in which the larger particles are agglutinated beads and the smaller particles are non-agglutinated beads.

23. A method for separating a bio-active material from other material which comprises adding beads which are able to agglutinate with the bio-active material to a

liquid containing the materials to be separated so as to cause agglutinated beads of the bio-active material. passing the liquid through an orifice which has an insert placed within it so as to form an annular gap of a size which prevents the agglutinated beads from contacting the gap and passing through it.

24. A method as claimed in claim 23 in which the beads are coated with an antigen or antibodies to the bio-active material.

25. A method as claimed in claim 23 or 24 in which the beads are beads of a diameter in the range of 0.5 μm to 10 μm

26. A method for measuring the concentration of a bio-active material in a liquid by adding to the liquid a known number of beads coated with an antigen or antibodies to the bio-active material to give a known concentration of coated beads, allowing the beads to agglutinate, separating the agglutinated beads from the non-agglutinated beads by the method claimed in claim 23 determining the concentration of non-agglutinated beads, calculating the concentration of agglutinated beads and determining the concentration of the bio-active material.

27. A method as claimed in claim 26 in which the material to be detected and counted by the method of the invention are proteins, viruses, bacteria, WBC, RBC and other similar material in the range of 0.01 μm to 40 μm.

28. A method as claimed in claim 26 or 27 in which the bio-active material/antigen components are antibodies to streptolysin B, C-reactive protein and rheomatoid factor.

29. A method for the detection of the fluorescence of particles in a liquid which method comprises passing liquid containing fluorescable particles through an orifice which has an optically transparent insert positioned in or adjacent to the orifice, is

passing the liquid over the insert, transmitting light through the liquid and detecting the fluorescence of particles in the liquid.

30. A method as claimed in claim 29 in which light is transmitted down a light guide to the insert and through the insert into the liquid and light emitted by the fluorescence of particles in the liquid passing through the orifice is transmitted back through the insert to a light guide to a detector.

31. A method as claimed in claim 29 or 30 in which the insert is placed as close to the orifice as possible without affecting the flow of liquid through the orifice to an unacceptable degree.

32. A method as claimed in claim 29, 30 or 31 in which the insert has a substantially flat end and light emitted from the particles as they pass the insert passes into the end of the insert and some of the particles hit the end of the insert.

33. A method as claimed in any one of claims 29 to 32 in which the insert is positioned in relation to the orifice so that substantially all the particles in a liquid passing through the orifice pass near enough to the end of the insert so that fluorescable particles fluoresce.

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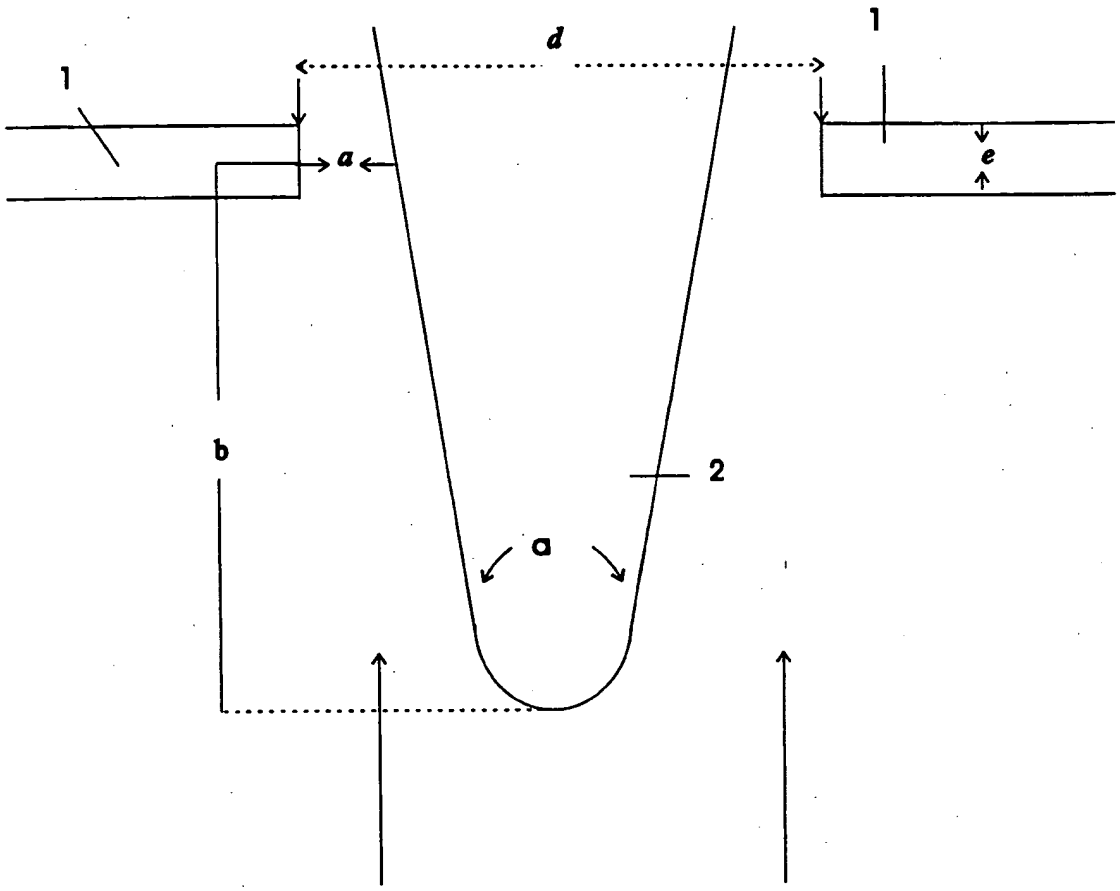

Fig. 1

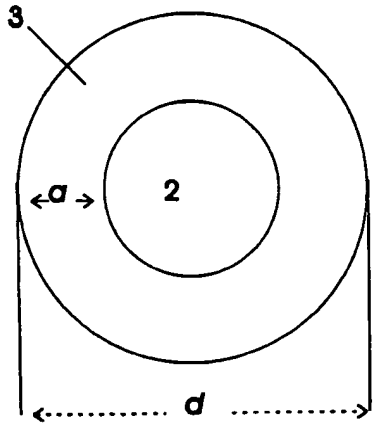

Fig. 2

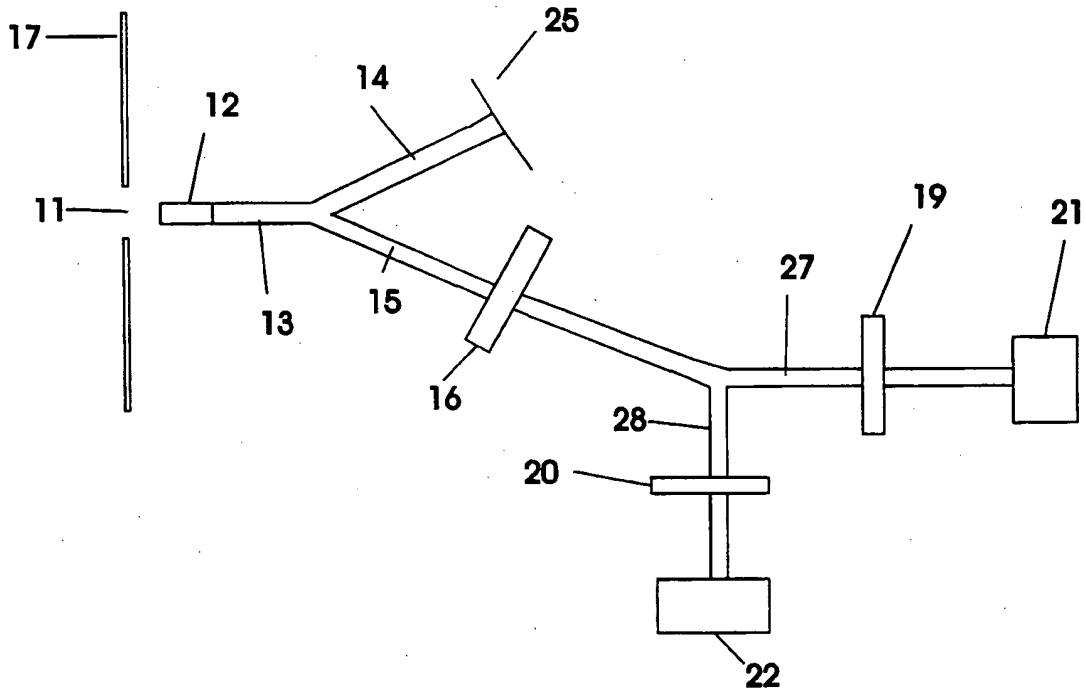

Fig. 3

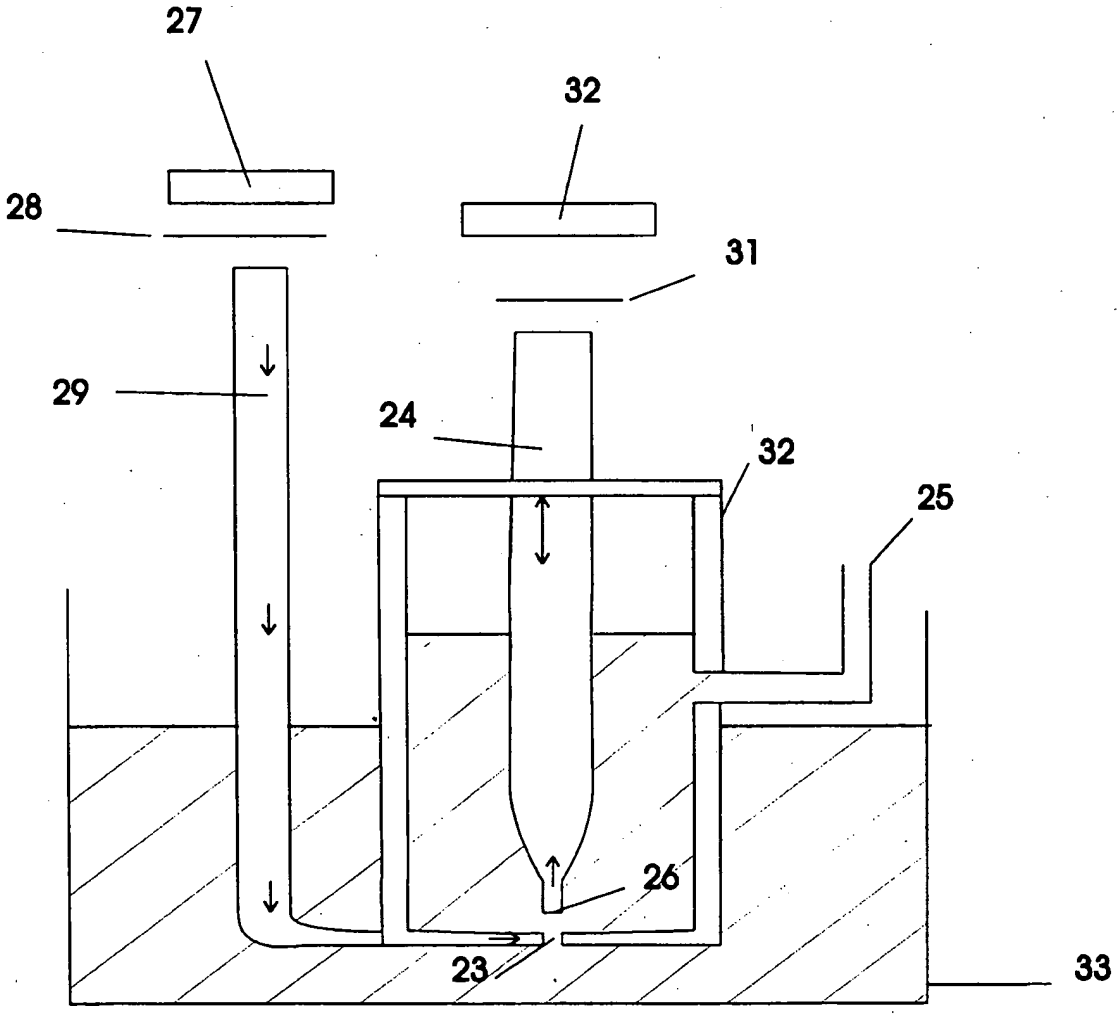

Fig.4

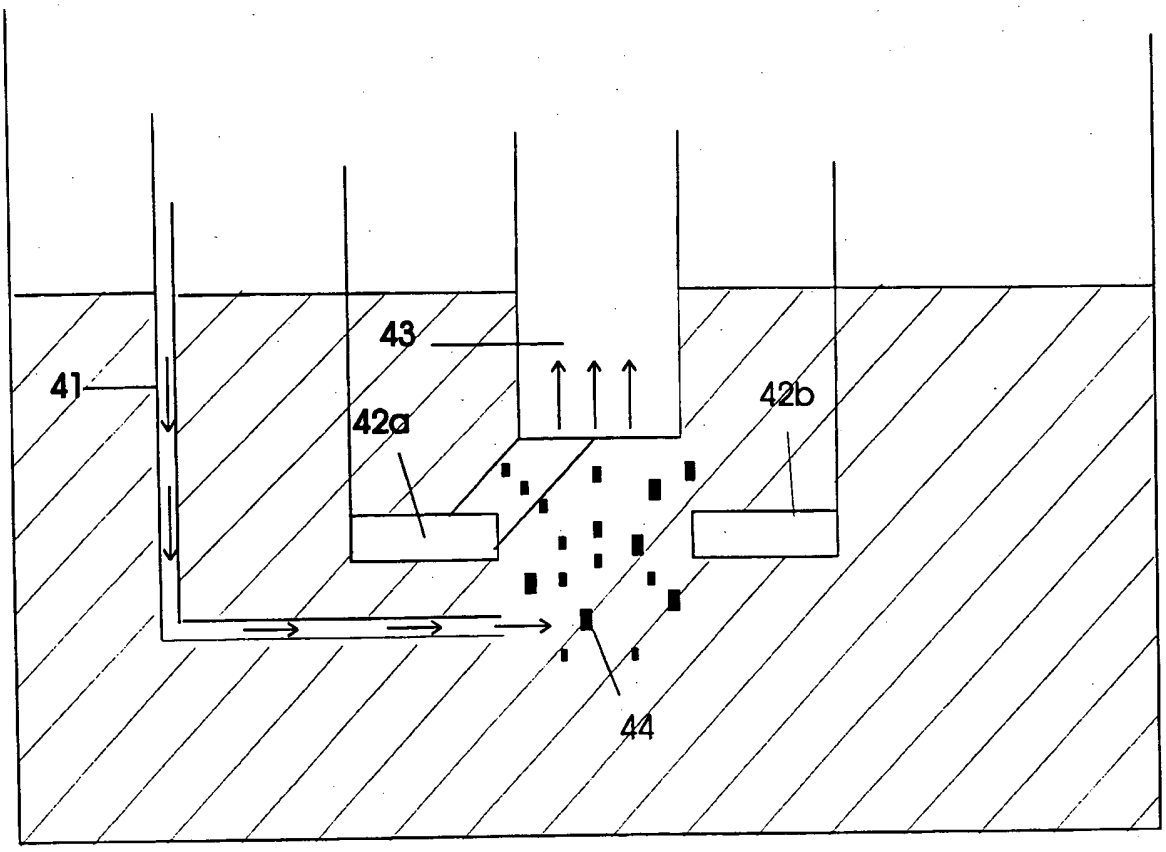

Fig. 5

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(71) Applicant and
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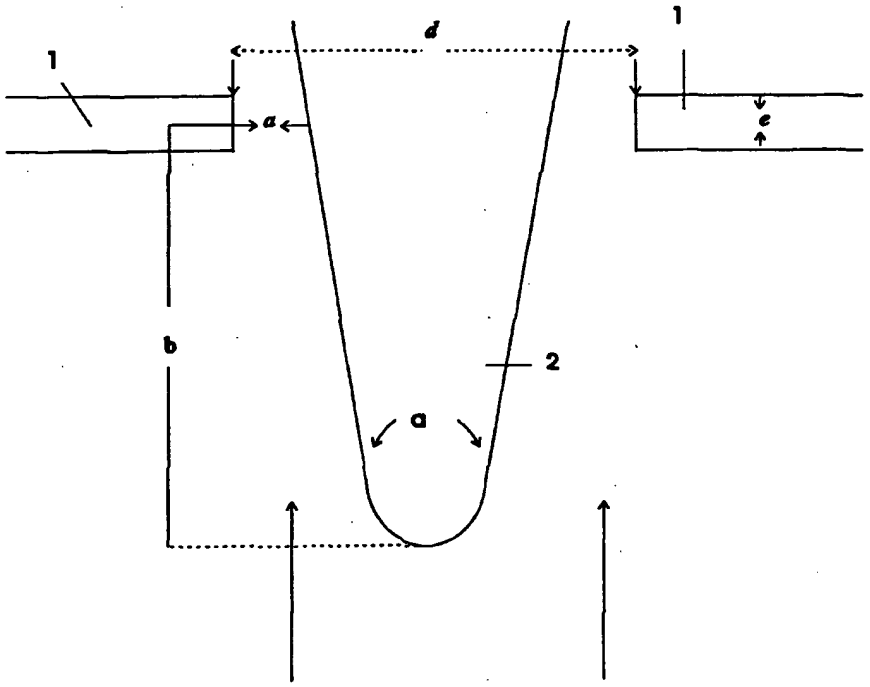
(54) Title: DEVICE AND METHOD FOR SEPARATION AND ANALYSIS OF SMALL PARTICLES



(57) Abstract: A device for separating particles in liquid suspension comprises a circular orifice (3) with an insert (2) so as form an annular gap (a), it is particularly useful in separating agglutinated particles and the device can also be used to count particles.

WO 01/22059 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 00/03559

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N15/12 B01D29/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 895 034 A (POOLE TRENT A) 23 January 1990 (1990-01-23) figure 6A	1
A	EP 0 242 971 A (HOLLEY JOHN ERNEST FOSTER) 28 October 1987 (1987-10-28) cited in the application the whole document	1-28
A	EP 0 262 568 A (VOITH GMBH J M) 6 April 1988 (1988-04-06) column 1, line 28-36	4,5
A	WO 96 29135 A (TOIT WALTERS FRANCOIS DU ;BROWN KEITH EDWIN FRANK (ZA)) 26 September 1996 (1996-09-26) page 10, line 3-9	21-28

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 January 2001	[illegible] 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Mueller, T

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ernational application No.
PCT/GB 00/03559

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-28

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-28

device and method of separating small particles and for analysing particlres in liquid suspensions, comprising a circular orifice and an insert to form an annular orifice

2. Claims: 29-33

method for detection of fluorescence of particles comprising and orifice and a transparent insert adjacent to the orifice

INTERNATIONAL SEARCH REPORT

Information on patent family members

Ir ational Application No
PCT/GB 00/03559

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 4895034 A	23-01-1990	WO 8901143 A	09-02-1989
EP 0242971 A	28-10-1987	DE 3787111 D	30-09-1993
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